Association of HIV-1 load and CD4 lymphocyte count with mortality among untreated African children over one year of age

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Objective: To examine the association of viral load and CD4 lymphocyte count with mortality among HIV-infected children over one year of age.

Design: A prospective study. HIV-infected children were enrolled during the first year of life and followed for more than 2 years at the Queen Elizabeth Central Hospital in Blantyre, Malawi (southeast Africa).

Methods: Morbidity and mortality information was collected every 3 months, and physical examination and blood testing (for viral level and CD4 cell percentage) were performed every 6 months. Kaplan–Meier analyses and proportional hazards models were used to estimate survival and to examine the association of primary predictors with mortality.

Results: Of 155 HIV-infected children originally enrolled, 115 (74%) had viral load testing and 82 (53%) had both viral load and CD4 cell percentage testing after their first year. Among children over one year of age, significant associations were found between mortality and the $\log_{10}$ viral load and CD4 cell percentage in both univariate and multivariate models. Independent of the CD4 cell value, a one unit $\log_{10}$ increase in HIV RNA level increased the hazard of child mortality by more than twofold. Children with low CD4 cell counts ($< 15\%$) and high viral loads ($\geqslant 250\,000$ copies/ml median value) had the worst survival; children with high CD4 cell counts ($\geqslant 15\%$) and low viral loads ($< 250\,000$ copies/ml) had the best survival.

Conclusion: As in developed countries, viral load and CD4 cell count are the main predictors of mortality among African children. Making these tests available adds to the challenges to be considered if antiviral therapies were to be adopted in these countries.

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Introduction

Studies on HIV perinatally infected children from developed countries have shown that the viral load and CD4 cell count are independently associated with HIV disease progression and death [1–4]. However, similar studies to document the prognostic utility of these indicators are not yet available from sub-Saharan Africa, where the number of children who become HIV infected is substantially higher than in industrialized countries. In this paper, we assess the association of viral load and CD4 lymphocyte percentage with mortality during the second and third years of life among a group of HIV-infected, untreated, children from Malawi. As in many developing countries, antiretroviral agents are not generally available in Malawi.

Methods

Between May and October 1995, 155 HIV-infected children were enrolled at the Queen Elizabeth Central Hospital in Blantyre, Malawi, and were prospectively followed to December 1997. These children, born in 1994, had initially participated in a birth canal cleansing intervention to reduce the perinatal transmission of HIV [5]. The HIV status of the baby was confirmed by polymerase chain reaction performed on dried blood spots [6] obtained at 6 and 12 weeks of age. The 155 children represented all HIV-infected children who attended the clinic between May and October 1995. They were enrolled after counselling the mother and obtaining informed consent. The median age of the child at enrolment was approximately 8 months. After enrolment, the children were seen every 3 months to collect morbidity and mortality data. The death of the child was directly reported to the clinic or ascertained by study workers when conducting home tracing for missed visits. Physical examination and the collection of blood samples were performed every 6 months. Routine clinical care (and referral when necessary) was provided in the study clinic for the children and their mothers by a designated clinical officer. The study was approved by the appropriate ethical committees in Malawi and the United States.

Laboratory tests

The HIV status of the infant was initially determined by DNA polymerase chain reaction using Roche Amplicor HIV-1 test (Roche Diagnostic Systems, Inc., Branchburg, NJ, USA) as described elsewhere [6]. The testing was performed at the Frederick Cancer Research Center, Frederick, MD, USA. Subsequently, whole blood collected on filter paper cards (Guthrie cards) from infected children was tested for quantitative HIV RNA level using a second generation isothermal nucleic acid silica-bound amplification assay (NucliSens HIV-1 RNA Q–T Kit; Organon Teknika, Durham, NC, USA) [7] adapted for use with dried blood spots [8]. These tests were conducted in Canada by a single laboratory that also participated in the AIDS Clinical Trials Group HIV-1 Virology Quality Assurance Programme. The results are expressed as copies of HIV-1 RNA per millilitre of blood. Only 115 of the 155 babies enrolled in this study had viral load measurements performed after the first year of life. The selection of these 115 samples for testing was random and independent of the CD4 cell count results and the clinical status of the baby.

The NucliSens RNA assay has been used in testing pediatric and adult specimens from several countries in southern Africa (including Malawi and South Africa) where subtype C predominates [9–11]. In those studies, a high correlation between the Roche Monitor and NucliSens assays was observed, and in adults, the results agreed with HIV serology. Comparison of the Roche Amplicor and NucliSens viral quantification assays using dried blood spots in pediatric subtype B groups have also shown no differences in the sensitivity or specificity of the two assays [12,13]. In the current study in Malawi, the NucliSens assay had a threshold sensitivity of 80 ($1.903 \log_{10}$) HIV-1 RNA copies per input volume [9].

CD4 cell values were determined on site using fresh peripheral blood samples collected after one year of age. It was not possible to perform the test on all children for various reasons related to the cost of the monoclonal antibodies, loss to follow-up, sickness of the baby and the mother's refusal to draw blood from the baby. Therefore, CD4 cell results were only available for 82 of the children who also had viral load measurements. White blood counts and differential (using a Coulter counter), and T lymphocyte subsets (using FACScan flow cytometry; Becton Dickinson, San Jose, CA, USA) were conducted as described in a previous report [14]. On the basis of recommendations in the United States [15] for children aged 1–5 years, severe, moderate and no immunosuppression were defined as CD4 cell values of less than 15%, 15–24%, and over 25%, respectively.

Statistical analysis

Descriptive, univariate and multivariate analyses were conducted to describe viral load and CD4 cell values and their associations with mortality. The analyses were restricted to deaths and laboratory test results (viral load and CD4 cell count) starting at 12 months and continuing to 36 months of the child's age. Arithmetic mean, median, range, and geometric mean were obtained for the viral load measurements. Logarithmic

(log_{10}) transformation of the viral load level was used as the predictor in the univariate and multivariate analyses. An 'index test' was defined as a viral load or CD4 cell count result obtained at or after 12 months of age. Only one CD4 cell test result was available for each of these 82 children after the first year of age. For viral load, the test result obtained closest to the CD4 cell result was used. Except in two children, the timing of the CD4 cell count and viral load blood draw was exactly the same. In one child, the CD4 cell sample was obtained at 20 months and the viral load at 22 months; in the other child the CD4 cell sample was obtained at 21 months and the viral load at 14 months. The median age of the child at the index test was 15 months (range 12–27 months).

The time of the index test represents time zero (or baseline value) for the survival analyses. The viral load and CD4 cell levels were used to predict child mortality after the index test. To estimate survival rates, Kaplan–Meier survival functions were fitted as the number of months the child survived after the index test was performed. Cox proportional hazards models were fitted to study covariate effects on the time from the index test to death. Besides the primary variables of interest (viral load and CD4 cell count), survival models were adjusted for the age of the child at the time of the index test, and for the child's HIV clinical category. On the basis of the 1994 Centers for Disease Control and Prevention (CDC) Revised Classification [16], children infected with HIV are classified into four mutually exclusive categories: 'N' not symptomatic; 'A' mildly symptomatic; 'B' moderately symptomatic; and 'C' severely symptomatic. The analysis of the predictors of mortality is restricted to the 82 children with both viral load and CD4 cell count measurements.

Results

Of the 155 children enrolled and followed during their second and third years of life, 115 (74%) had available viral load measurements, and of these 115 children, 82 (71%) also had CD4 cell count results. Table 1 shows the characteristics of these three groups of children and of their mothers. The 82 children were similar to those with no or only partial testing.

Table 2 shows the descriptive statistics at baseline of viral load measurements, CD4 cell percentage values and the clinical classification of HIV disease among the children who died (N = 29) and those who were alive (N = 53). The median and the geometric mean viral loads were, respectively, 1.6- and 1.9-fold higher among children who subsequently died compared with those who did not die. Likewise, the mean and median CD4 cell percentages were lower, and the frequency of category C disease was higher in children who died compared with those who were censored (Table 2).

Table 1. Characteristics of the study population[a].

	Total no. of HIV -positive babies enrolled	Babies enrolled: have viral load test	Babies enrolled: have both viral load and CD4% tests
N	155	115	82
Died (%)	34	30	35
Median duration of follow-up (months)	12	12	12
Person-years of follow-up	153	121	85
Mortality rate per 100 person-years	34.0	28.9	34.1
Median age at enrolment (months)	8.8	8.8	8.4
Median age of 'index test' (months)[b]	N/A[c]	15	15
Mean birth weight (g)	2797	2831	2814
Male sex (%)	49.4	45.2	46.3
HIV clinical category (%)[d]:			
N or A	3.9	2.6	2.5
B	88.3	88.6	85.4
C	7.8	8.8	12.1
Viral load copies/ml:			
Mean	N/A	508 783	494 672
Range	N/A	2900–4 400 000	8000–4 400 000
Median	N/A	240 000	250 000
Geometric mean	N/A	182 810	205 589
Mean maternal age (years)	24.2	24.5	24.1
Mean parity	2.8	2.8	2.6
Maternal education: illiterate (%)	8.4	7.0	9.8

[a]Child mortality, follow-up and viral load results refer to estimates after the first year of age.
[b]Age of the child when viral load and CD4 cell test samples were obtained.
[c]Not applicable: viral load and CD4 cell tests not performed on all children.
[d]Centers for Disease Control and Prevention 1994 revised clinical classification [16]; see Methods.

Table 2. Viral load, CD4 cell percentage and HIV clinical category among the 82 children included in the analysis.

	Died N = 29	Censored[a] N = 53	*P* value[b]
Viral load (copies/ml)c			
Log_{10} load			0.011
Mean	584 690	445 417	
Range	23 000–2 100 000	8000–4 400 000	
Median	310 000	190 000	
Geometric mean	309 029	163 681	
Less than median	34.5%	58.5%	
Median or greater	65.5%	41.5%	
CD4 cell percentage[c]			0.016
Mean	16.8	20.2	
Range	6.0–41.0	4.0–40.0	
Median	14.0	19.0	
< 15	51.7%	22.6%	
15–24	31.0%	47.2%	
25+	17.2%	30.2%	
Clinical category[d]			0.100
N or A	3.5%	1.9%	
B	75.9%	90.6%	
C	20.6%	7.5%	

[a]Censored alive at 36 months of age (study closure) or lost to follow-up.
[b]From univariate proportional hazards model (viral load and CD4 cell percentage as continuous covariates; clinical category as binary: C versus N/A/B as reference); see Results.
[c]Viral load and CD4 cell count determined after year one of age.
[d]Based on Centers for Disease Control and Prevention 1994 revised classification system [16]; see Methods.

Univariate proportional hazards models, which compared the differences between children who died and those alive, showed statistically significant differences in viral load and CD4 cell percentage values (Table 2). However, no significant differences were found in clinical HIV disease (severely symptomatic versus not, mildly or moderately symptomatic).

Kaplan–Meier survival probabilities stratified by baseline median viral load, CD4 cell percentage categories and these two variables combined are shown in Fig. 1. Children with median or greater viral loads (250 000 copies/ml) had significantly lower survival rates during follow-up after time zero compared with children with viral load levels lower than the median ($P < 0.02$; log-rank test). For example, 52% of those with viral loads of 250 000 copies/ml or greater survived 9 months beyond the index test compared with 80% of those with viral loads of less than 250 000 copies/ml. Children with CD4 cell counts of less than 15% had significantly lower survival rates compared with children with CD4 cell counts of 15–24% or 25% or greater ($P < 0.0003$; overall log-rank test). Among children with CD4 cell counts of 15% or greater, CD4 cell counts of 15–24% and CD4 cell counts of 25% or greater, approximately 80% survived 9 months beyond the index test compared with 40% of children with CD4 cell counts of less than 15%. Survival probabilities between children with CD4 cell values of 15–24% and CD4 cell counts of 25% or greater did not differ statistically. The stratification of survival by both the median viral load and the CD4 cell count (< 15 versus ⩾ 15%) showed that children with high viral loads (⩾ 250 000) and low CD4 cell counts (< 15%) had the worst survival (approximately 86% died within 9 months). In comparison, children with low viral loads (< 250 000) and high CD4 cell counts (⩾ 15%) had the best survival (85% survived > 18 months) (Fig. 1).

There was a significant association between child mortality after the first year of age and log_{10} viral load and the CD4 cell percentage when these variables were included separately as continuous variables in proportional hazards models (i.e. univariate analysis; Table 2). These significant associations persisted after including simultaneously the viral load (as a continuous variable) and the CD4 cell percentage (as continuous or categorical) in a multivariate proportional hazards model (Table 3). The age of the child in months was not significant in univariate or multivariate analyses (e.g. risk ratio per month 0.97, $P = 0.99$ in the multivariate analysis), and did not change the magnitude of association of the primary variable with child mortality. Similarly, after adjustment for viral load and CD4 cell percentage, there were no significant associations between child mortality and other covariates, including the clinical category of HIV disease (C versus B/A/N), the death of the mother (five died), and the birthweight of the child. Therefore, we did not include these covariates in the final models (Table 3).

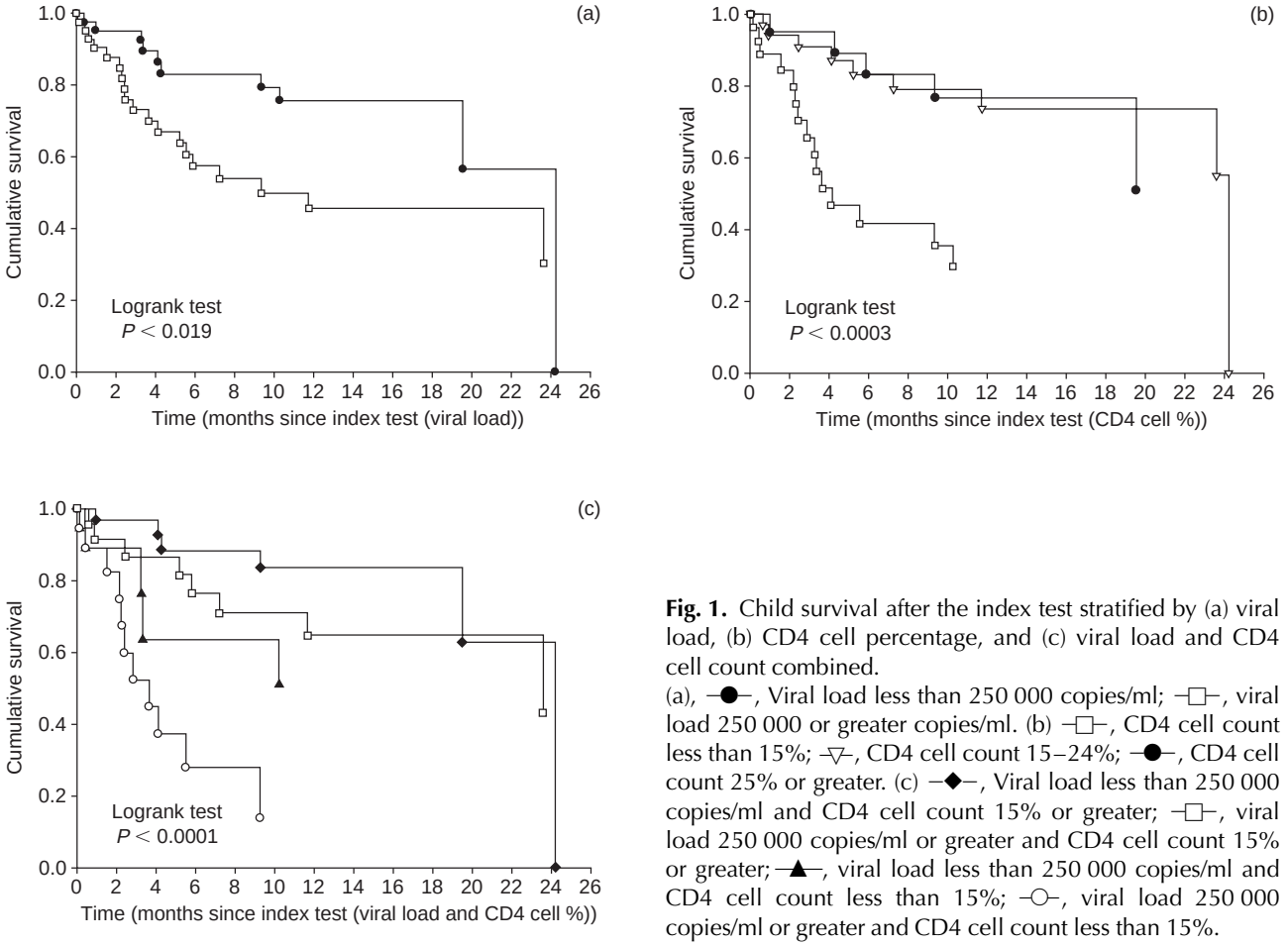

Fig. 1. Child survival after the index test stratified by (a) viral load, (b) CD4 cell percentage, and (c) viral load and CD4 cell count combined.
(a), —●—, Viral load less than 250 000 copies/ml; —□—, viral load 250 000 or greater copies/ml. (b) —□—, CD4 cell count less than 15%; —▽—, CD4 cell count 15–24%; —●—, CD4 cell count 25% or greater. (c) —◆—, Viral load less than 250 000 copies/ml and CD4 cell count 15% or greater; —□—, viral load 250 000 copies/ml or greater and CD4 cell count 15% or greater; —▲—, viral load less than 250 000 copies/ml and CD4 cell count less than 15%; —○—, viral load 250 000 copies/ml or greater and CD4 cell count less than 15%.

Table 3. Association of viral load and CD4 cell percentage with child mortality.

	Unadjusted RR (95% CI)	Adjusted RR (95% CI) *P* value	Adjusted RR (95% CI) *P* value
		Model 1[a]	Model 2[a]
Log_{10} viral load (continuous)	2.45 (1.23–4.88)	2.11 (1.03–4.32) 0.040	2.23 (1.03–4.82) 0.042
CD4 cell percentage (continuous)[b]	0.72 (0.55–0.94)	0.77 (0.58–1.01) 0.057	
CD4 cell percentage:			
< 15	4.18 (1.99–11.73)		3.21 (1.11–9.24) 0.031
15–24	0.93 (0.89–0.96)		0.79 (0.25–2.53) 0.690
25+	1.00		1.00

CI, 95% confidence interval; RR, risk ratio.
[a]Model 1 includes log_{10} viral load and CD4 cell percentage as continuous variables; Model 2 includes log_{10} viral load as a continuous variable and CD4 cell percentage as a categorical variable. The age of the child at the time of the index test (CD4 cell count or viral load) was not significantly associated with mortality in the univariate or multivariate analyses.
[b]Per 5% increase in CD4 cell percentage.

Discussion

Our analyses confirm that high viral load and low CD4 lymphocyte percentage are independently and significantly associated with mortality among African children who are perinatally HIV infected. These findings are consistent with reports from developed countries [1,17,18]. Several aspects of this study should be highlighted. First, the study provides data on viral load and CD4 cell values from sub-Saharan Africa, where such information is not generally available. The data also pertain to HIV subtype C, the predominant clade in Malawi and southern Africa [19]. Second, the data suggest that the value of viral load and CD4 lymphocyte measures are as important in developing countries as in developed countries, despite the vast differences in

environmental exposure and nutritional factors. Third, the association of viral load and CD4 cell count with mortality was assessed after the first year of life. Therefore major risk factors that contribute to infant mortality, such as low birthweight, are excluded. Finally, the investigation of viral and immunological factors in this study was not confounded by the use of antiretroviral agents at any time.

As in other studies [1,2,4], children with severe immunosuppression (CD4 cell count $< 15\%$) and high viral loads ($>$ median) had the worst outcome compared with children without severe immunosuppression and high viral loads (Fig. 1), suggesting a cumulative effect. Both viral load and CD4 cell percentage were independently associated with mortality in the multivariate proportional hazards model (Table 3). The effect of the CD4 cell percentage was unchanged when this variable was considered as categorical or continuous (per 5% units increase). For example, after taking into account the level of viral load, the mortality hazard for children with severe immunosuppression (CD4 cell count $< 15\%$) was more than threefold compared with children with normal CD4 lymphocyte values ($> 25\%$). Similarly, when considered as a continuous variable, a 5% increase in CD4 cell value decreased the hazard of mortality by approximately 23% (Table 3). Irrespective of the CD4 cell percentage definition, a one unit $\log_{10}$ increase in HIV RNA level increased the hazard of child mortality by more than twofold (models 1 and 2; Table 3).

The utility of the viral load and CD4 cell percentage to predict mortality was assessed after the index test in a 'time-fixed' analysis (i.e. covariates are defined at baseline). Because the follow-up time was relatively short (a median of 12 months after the first year of age), the viral load and CD4 cell count could be predictive of short-term survival in this study. Other studies, however, have shown that viral load is an important short- and long-term predictor of disease progression after adjusting for immunological and clinical status [18].

We adjusted for the age of the child at the index test and for the clinical category of HIV disease when assessing the association of viral load and CD4 cell percentage with child mortality. Neither age nor clinical category was significantly associated with mortality in either univariate or multivariate analyses. The lack of mortality association with the child's age (once the viral load and CD4 cell count have been adjusted for) has also been observed in other studies [2,4]. This finding has a practical implication. It suggests that after a child has survived the first year, a measurement of viral load or CD4 cell percentage at any age could be directly predictive of future survival, without having to adjust for the age of the child.

Comparison between the entire sample of HIV-infected children enrolled in the study, those with only viral load results, and those with both viral load and CD4 cell percentage results showed no major differences in several factors (Table 1). Therefore, it is unlikely that our results are biased as a result of the selection of the 82 children included in the current analyses. In addition, the selection of blood samples for viral load testing was independent of CD4 cell results. The testing of CD4 cell counts was performed locally in Malawi. The laboratory performing the viral load measurements in Canada was not aware of the matching CD4 cell results.

Conclusion

This study shows that viral levels and CD4 cell percentage both add helpful prognostic information for the management of children in developing countries. In developed countries the use of viral load and CD4 cell values has been recommended to guide the initiation of antiretroviral therapy and to monitor treatment response. Antiretroviral agents are not yet the standard of care in Africa. The inability to measure these predictors adds to the complexity of adopting therapies in areas where the existing infrastructure does not provide these tests.

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